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ORIGINAL ARTICLE *Infertility*

A prospective cohort study of endometriosis and subsequent risk of infertility

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STUDY QUESTION: Is there a temporal relationship between endometriosis and infertility?

SUMMARY ANSWER: Endometriosis is associated with a higher risk of subsequent infertility, but only among women age <35 years.

WHAT IS KNOWN ALREADY: Endometriosis is the most commonly observed gynecologic pathology among infertile women undergoing laparoscopic examination. Whether endometriosis is a cause of infertility or an incidental discovery during the infertility examination is unknown.

STUDY DESIGN, SIZE, DURATION: This study included data collected from 58 427 married premenopausal female nurses <40 years of age from 1989 to 2005, who are participants of the Nurses' Health Study II prospective cohort.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Our exposure was laparoscopically confirmed endometriosis. Multivariate Cox proportional hazards regression models were used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) for infertility risk (defined as attempting to conceive for >12 months) among women with and without endometriosis.

MAIN RESULTS AND THE ROLE OF CHANCE: We identified 4612 incident cases of infertility due to any cause over 362 219 person-years of follow-up. Compared with women without a history of endometriosis, women with endometriosis had an age-adjusted 2-fold increased risk of incident infertility (HR = 2.12, 95% CI = 1.76–2.56) that attenuated slightly after accounting for parity. The relationship with endometriosis was only observed among women <35 years of age (multivariate HR <35 years = 1.77, 95% CI = 1.46–2.14; multivariate HR 35–39 years = 1.20, 95% CI = 0.94–1.53; *P*-interaction = 0.008). Risk of primary versus secondary infertility was similar subsequent to endometriosis diagnosis. Among women with primary infertility, 50% became parous after the endometriosis diagnosis, and among all women with endometriosis, 83% were parous by age 40 years.

LIMITATIONS, REASONS FOR CAUTION: We did not have information on participants' intentions to conceive, but by restricting the analytic population to married women we increased the likelihood that pregnancies were planned (and therefore infertility would be recognized). Women in our cohort with undiagnosed asymptomatic endometriosis will be misclassified as unexposed. However, the small proportion of these women are diluted among the >50 000 women accurately classified as endometriosis-free, minimizing the impact of exposure misclassification on the effect estimates.

WIDER IMPLICATIONS OF THE FINDINGS: This study supports a temporal association between endometriosis and infertility risk. Our prospective analysis indicates a possible detection bias in previous studies, with our findings suggesting that the infertility risk posed by endometriosis is about half the estimates observed in cross-sectional analyses.

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Key words: endometriosis / infertility / prospective cohort studies / bias / epidemiology

Introduction

Endometriosis is a benign disease in which endometrial-like tissue persists outside of the uterus. Pelvic structures are most commonly affected, but endometriosis can involve extrauterine organs as distant as the lung (Azizad-Pinto and Clarke, 2014). Patients typically present with pelvic pain, infertility and/or an ovarian endometriosis cyst (Barbieri and Missmer, 2002). An estimated 5–10% of the general female population are affected by endometriosis, with an even higher prevalence (~20–30%) among women with infertility, thus supporting the potential for a causal relationship between endometriosis and infertility (Wheeler, 1989; Tanahatoe *et al.*, 2003; Giudice and Kao, 2004; Missmer *et al.*, 2004; Giudice *et al.*, 2010). A complexity in investigating the temporal relationship between endometriosis and infertility, however, is that among infertile women, their endometriosis can be asymptomatic, and thus, is diagnosed through the course of an infertility evaluation (Barbieri and Missmer, 2002).

While endometriosis is frequently identified during an infertility diagnosis, a causal link has yet to be demonstrated. Since surgical visualization is the gold standard for a definitive diagnosis (Pardanani and Barbieri, 1998), prevalence and incidence estimates among infertile women are presumably an overestimate of those expected in the general population (Vercellini *et al.*, 2014). It is possible that women with otherwise 'asymptomatic' endometriosis secondary to the primary causes of infertility are over-represented, and these women would not have received a laparoscopic diagnosis of endometriosis had they not attempted to become pregnant (Cramer and Missmer, 2002; Missmer *et al.*, 2004). In addition, spontaneous pregnancy rates among endometriosis patients did not differ by hormonal treatment of endometriosis in high-quality clinical trials (Barbieri and Missmer, 2002; Loverro *et al.*, 2008; Alborzi *et al.*, 2011; Alkatout *et al.*, 2013). Hormonal treatment may not completely resolve endometriotic lesions in >50% of women; recurrence of symptoms occurring within 1 year is common (Mettler *et al.*, 2014). This may contribute to the lack of improvement in infertility. Furthermore, surgical removal of pelvic lesions provides only moderate reproductive benefit (Marcoux *et al.*, 1997; Vercellini *et al.*, 2014).

Despite the poorly understood relationship between endometriosis and infertility, some clinicians promote fertility preservation, for example, egg freezing, among reproductive age women with endometriosis (Somigliana *et al.*, 2015). Given the longitudinal nature of the Nurses' Health Study II (NHS II), we are able to prospectively evaluate the temporal relationship between a history of laparoscopically confirmed endometriosis and infertility among premenopausal women.

Materials and Methods

Study population

The NHS II is an ongoing prospective cohort following 116 430 female US registered nurses who were 24–44 years of age at enrollment in 1989. At baseline and biennially thereafter, participants completed self-administered questionnaires to capture detailed information on a variety of lifestyle and reproductive characteristics and to update health-related outcomes. Follow-up for each questionnaire cycle was >90%.

We limited our study population to premenopausal married women <40 years of age ($n = 68\,003$) since this group of women has been reported to be much more likely to actively plan a pregnancy and therefore more likely to report infertility when compared with women who are unmarried and/or 40+ years of age (Chandra *et al.*, 2014). Data from our cohort appear consistent with these assumptions in that the proportion of premenopausal women reporting incident infertility consistently was greater among married women and those younger than 40 years of age in all questionnaire cycles (data not shown). At baseline, we sequentially excluded women with a history of infertility ($n = 13\,622$), cancer (except non-melanoma skin cancer; $n = 404$) and a report of endometriosis never confirmed by laparoscopy ($n = 349$). Premenopausal women <40 years of age who were not married at baseline contributed person-time to the analysis if and when their marital status changed to married in subsequent questionnaires ($n = 4799$). Over 99% of premenopausal married women <40 years of age reported currently living with their spouse.

Ethical approval

The NHSII protocol was approved by the Institutional Review Board of the Partners Health Care System, Boston, MA, USA.

Endometriosis exposure assessment

Women were asked on each biennial questionnaire whether they had received a physician diagnosis of endometriosis. Participants responding 'yes' then indicated the year of diagnosis and whether it had been confirmed by laparoscopy (the gold standard for diagnosis) (Duleba, 1997; Pardanani and Barbieri, 1998). Self-reported endometriosis was previously validated among 200 randomly selected NHS II participants who reported an incident endometriosis diagnosis on the 1993 questionnaire. For those women where medical records were available, the diagnosis of endometriosis was confirmed in 96% of women reporting laparoscopically confirmed endometriosis, but confirmed in only 54% of women without laparoscopic confirmation (Missmer *et al.*, 2004). Due to the high potential for misclassification of self-reported endometriosis without laparoscopic confirmation, we restricted our exposure definition to laparoscopically confirmed endometriosis.

Once a woman reported laparoscopically confirmed endometriosis, she remained 'exposed' (i.e. was considered to have endometriosis) through the end of follow-up. To maintain prospective temporality between exposure and outcome, women who reported both endometriosis and infertility on the same questionnaire cycle ('concurrent') were not classified as exposed. We did this to minimize bias from the likelihood of women with incident infertility to receive an endometriosis diagnosis (i.e. their endometriosis would have gone undetected if not for their infertility examinations). However, in order for our statistical models to capture the cross-sectional influence of classifying a woman with a concurrent diagnosis as exposed, as all previous studies of this topic have to date, we modified our definition of the exposure period in such a way that women were treated as if endometriosis had been present at least 2 years prior to the endometriosis report. We

conducted an additional sensitivity analysis in which women with a concurrent diagnosis were censored at the time of the concurrent diagnosis.

Infertility outcome ascertainment

Participants were asked on each biennial questionnaire through 2001, and every 4 years thereafter, whether they had tried to become pregnant for more than 1 year without success. Those responding 'yes' were asked to indicate whether their inability to conceive was attributed to tubal blockage, ovulatory disorder, endometriosis, cervical mucous factor, spousal factor, cause not investigated, cause not found and/or due to another reason. Total infertility was defined as infertility from any cause. Within this cohort, self-reported infertility was validated in a subset of 100 randomly selected women who reported ovulatory infertility; 95% of self-reports were confirmed through medical record review (Rich-Edwards *et al.*, 1994).

Covariate data

On the 1989 baseline questionnaire, participants reported a number of characteristics, including their height, current weight, weight at age 18 years, physical activity, health screening behavior, smoking status, analgesic use, age at menarche, menstrual cycle length and pattern between ages 18 and 22 years, oral contraceptive (OC) use, sterilization procedures, marital status, parity (number of pregnancies lasting ≥6 months), menopausal status, personal history of diabetes, cardiovascular disease or cancer, and their race or ethnicity. All time-varying characteristics were updated every 2 years, except for physical activity and marital status, which were updated every ~4 years. Current living arrangement, which was first reported on the 1993 questionnaire, was updated every 4 years. Nurses' birthweight and a personal history of hirsutism were reported on the 1991 questionnaire.

Health screening behaviors included any reports of following: physical exam, breast exam, mammogram, Pap smear, bimanual pelvic exam, ovarian ultrasound, colonoscopy, sigmoidoscopy and/or testing of fasting blood sugar. BMI was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). From the frequency, duration and intensity of reported non-leisure activities, we calculated total metabolic equivalent (MET) hours of activity per week (Ainsworth *et al.*, 1993; Colditz *et al.*, 2003). Women who had used OC for ≥2 months were classified as ever-users (Charlton *et al.*, 2014). Women who indicated 'Southern European/Mediterranean', 'Scandinavian' or 'other Caucasian', but not 'African American', 'Hispanic' or 'Asian' were grouped as whites. A validated food frequency questionnaire (Giovannucci *et al.*, 1991; Rimm *et al.*, 1992; Feskanich *et al.*, 1993) captured usual dietary intake starting in 1991 and every 4 years thereafter. The Alternative Healthy Eating Index (AHEI) score was calculated for each participant to measure their adherence to a dietary pattern based on healthful foods and nutrients (Chiuve *et al.*, 2012). Values for 1989 were back filled using 1991 data. AHEI scores were updated as a cumulative average every 4 years to better reflect long-term intake and decrease measurement error (Hu *et al.*, 1999).

Statistical analyses

Person-time was calculated for each eligible participant from the return date of the baseline questionnaire or the questionnaire in which the woman reported being married, whichever happened first, until the questionnaire in which the woman first reported infertility due to any cause, reached 40 years of age, reached menopause or underwent a sterilization procedure (herself or her partner), was diagnosed with cancer (except non-melanoma skin cancer), returned her last questionnaire, had died of any cause or reached end of follow-up (1 June 2005—the questionnaire cycle in which the youngest woman was now age 40+ years), whichever occurred first. Women who reported physician-diagnosed endometriosis with no laparoscopic confirmation were censored at the time of that report, but were allowed to re-enter the analysis population with their interim person-time if they subsequently reported laparoscopic confirmation on a questionnaire.

Cox proportional hazards regression models, with age in months as the time scale and stratified by 2-year questionnaire cycle, were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) of infertility risk for women with a history of laparoscopically confirmed endometriosis versus those without. All time-varying covariates were updated with each questionnaire. We observed a significant interaction by age, so we included an age interaction term in our final models. Multivariate models were additionally adjusted for established infertility risk factors: age at menarche (<12, 12–13, 14+ years), menstrual cycle length between ages 18 and 22 years (<26, 26–31, 32–39, 40–49, 50+ days or irregular pattern), current BMI (<18.5, 18.5–22.4, 22.5–24.9, 25.0–29.9, 30+ kg/m^2), total physical activity (<3, 3–8.9, 9–17.9, 18–26.9, 27–41.9, 42+ METS/week), smoking status (never, past, current) and AHEI diet score (quintiles). Missing indicators were included for model covariates as needed (Miettinen, 1985). We added parity (continuous) separately to the multivariate model, because it may be an intermediate in the pathway of the endometriosis–infertility relationship (Weinberg, 1993; Howards *et al.*, 2007). We also considered menstrual cycle pattern, hirsutism, participant's birthweight, race, household income, husband's education, BMI at age 18 years, alcohol consumption, OC use, any analgesic use, health screening behavior, personal history of cardiovascular disease and personal history of diabetes as potential confounders. These covariates did not change estimates by >10% and, thus, were not included in the final models.

In sensitivity analyses, we included all premenopausal women <40 years of age regardless of marital status. We also conducted likelihood ratio tests to assess effect modification stratified by selected factors. To distinguish between primary (nulliparous at first infertility report) versus secondary (parous at first infertility report) infertility, we stratified the analysis by whether a woman was nulliparous at the time of infertility diagnosis. Secondly, given the well-recognized age-related decline in fertility (American College of Obstetricians and Gynecologists Committee on Gynecologic Practice and Practice Committee, 2014), we estimated the risk separately for women <35 versus 35–39 years of age. Thirdly, since a substantial proportion of infertility may be attributable to excess adiposity (Rich-Edwards *et al.*, 2002; Talmor and Dunphy, 2015), we also stratified the analysis by BMI (<25 versus 25+ kg/m^2). All *P*-values are two-sided using an alpha level of 0.05 to indicate statistical significance. We used SAS Version 9.3 software (SAS Institute, Cary, NC, USA) for all analyses.

Results

Among the 58 427 eligible women included in our analysis, 3537 (6%) reported a diagnosis of laparoscopically confirmed endometriosis. Of these women, 74% were parous prior to endometriosis diagnosis. Among women with primary infertility, 50% became parous after the endometriosis diagnosis, and among all women with endometriosis, 83% were parous by age 40 years. Of these women, 15% reported ever use of clomiphene or gonadotrophin to stimulate ovulation, and 2% reported ever use of IVF. While we are actively collecting infertility treatment data, presently, we are not able to link specific infertility treatments to timing of endometriosis diagnosis without introducing misclassification.

At the midpoint of follow-up (1995), women with endometriosis were more likely to have had a younger age at menarche, irregular menstrual patterns in young adulthood, to ever have used OCs, report current use of analgesics greater than or equal to two times per week, health screening behaviors, to be a current smoker, and to be nulliparous compared with their counterparts (Table I). BMI at age 18 years, current BMI

Table I Age-standardized characteristics of participants in the Nurses' Health Study II at the midpoint of follow-up (1995).

	Laparoscopically confirmed endometriosis	
	No (n = 22 581)	Yes (n = 658)
Age (years)[a]	36.2 (2.5)	36.5 (2.4)
Age at menarche (years)		
Menarche <11, %	22	26
Menarche 12–13, %	58	57
Menarche ≥14, %	20	17
Regular menstruation at ages 18–22, %	76	71
Irregular menstruation at ages 18–22, %	21	24
Hirsutism, %	1	2
Nulliparous, %	9	15
Ever OC use, %	86	95
BMI at age 18 (kg/m^2)	21.0 (2.9)	21.0 (3.0)
BMI <18.5 at age 18, %	14	16
BMI 18.5 to <22.5 at age 18, %	64	61
BMI 22.5 to <25.0 at age 18, %	14	15
BMI 25.0+ at age 18, %	8	8
Current BMI (kg/m^2)	24.7 (5.1)	25.1 (5.1)
BMI <18.5, %	2	2
BMI 18.5 to <22.5, %	36	34
BMI 22.5 to <25.0, %	23	22
BMI 25.0 to <30.0, %	22	23
BMI 30.0+, %	13	16
Total physical activity (METS/week)	21.8 (27.8)	23.0 (34.3)
Never smoker, %	72	66
Former smoker, %	20	20
Current smoker, %	8	13
AHEI score	47.4 (9.6)	46.8 (9.8)
Alcohol intake (grams/day)	3.1 (5.8)	2.8 (6.0)
Any analgesic 2+ or more times per week, %	39	46
Any screening behavior, %	84	88
White, %	93	95

Values are means (SD) or percentages and are standardized to the age distribution of the study population.
OC, oral contraceptive; METS, metabolic equivalent task score; AHEI, alternative health eating index.
[a]Value is not age-adjusted.

and total physical activity were similar between the two groups. Between return of the baseline questionnaire and 1 June 2005 (the last date at which women in the cohort were younger than 40 years), we identified 4612 incident cases of infertility due to any cause over 362 219 person-years of follow-up.

Table II History of laparoscopically confirmed endometriosis and risk of incident infertility among married women in Nurses' Health Study II from 1989 to 2005.

Laparoscopically confirmed endometriosis	No	Yes	P-value
Cases, N	4429	183	
Person-years	353 490	8730	
Age-adjusted HR (95% CI)	1.00 (ref)	2.12 (1.76–2.56)	<0.0001
Multivariate-adjusted HR[a] (95% CI)	1.00 (ref)	2.11 (1.74–2.54)	<0.0001
Multivariate-adjusted HR[b] (95% CI)	1.00 (ref)	1.78 (1.48–2.15)	<0.0001

HR, hazard ratio; CI, confidence interval.
[a]Adjusted for age (months), age interaction term, questionnaire cycle, age at menarche, menstrual cycle length between ages 18 and 22 years, current BMI, total physical activity, smoking status, cumulatively updated average AHEI diet score.
[b]Additionally adjusted the multivariate model for parity (number of children).

Laparoscopically confirmed endometriosis was reported by 751 (16%) infertile women. Women with a history of endometriosis had a 2-fold greater risk of infertility after adjusting for age (HR = 2.12, 95% CI = 1.76–2.56; $P < 0.0001$; Table II). Adjusting for all measured established risk factors, including age at menarche, menstrual cycle length between ages 18 and 22 years, current BMI, total physical activity, smoking status, and AHEI diet score, did not impact the effect estimate (HR = 2.11, 95% CI = 1.74–2.54; $P < 0.0001$). The inclusion of parity in the multivariate model attenuated the estimate, but a history of endometriosis remained significantly associated with a 78% greater risk of infertility (95% CI = 1.48–2.15; $P < 0.0001$). Effect estimates were similar when unmarried premenopausal women <40 years of age were included in analyses (data not shown). Endometriosis was not associated with greater risk of other infertility types (e.g. ovulatory infertility), although sample sizes were small (e.g. $n = 28$ cases of ovulatory infertility, $n = 11$ cases of male factor infertility among women with endometriosis).

Risk estimates did not differ by primary versus secondary infertility (P-interaction = 0.54; Table III). The relation between endometriosis and infertility was modified by age and BMI. Specifically, endometriosis was related to infertility among women <35 years of age in the multivariate model adjusted for parity (HR = 1.77, 95% CI = 1.46–2.14), but not among women 35 and older (HR = 1.20, 95% CI = 0.94–1.53; P-interaction = 0.008). In addition, endometriosis was related to a higher infertility risk among lean women (HR = 1.96, 95% CI = 1.59–2.42), but not among overweight/obese women (HR = 1.30, 95% CI = 0.83–2.05; P-interaction = 0.02).

In our sensitivity analysis in which women concurrently reported endometriosis and infertility in the same questionnaire cycle ($n = 223$) were considered exposed (i.e. by assuming endometriosis was present for at least 2 years prior to endometriosis report) rather than unexposed (Table IV) to endometriosis, we observed the association between endometriosis and infertility nearly doubled in the multivariate-adjusted model (HR = 3.23, 95% CI = 2.85–3.67). With this modified exposure definition, we also observed a somewhat stronger association with

Table III History of laparoscopically confirmed endometriosis and risk of incident infertility among married women in Nurses' Health Study II from 1989 to 2005, stratified by primary versus secondary infertility, age and BMI categories

Laparoscopically confirmed endometriosis	Primary infertility			Secondary infertility			*P*-het
	No	Yes	*P*-value	No	Yes	*P*-value	
Cases, *n*	2132	101		2297	82		
Person-years	42 606	1409		310 883	7321		
Age-adjusted HR (95% CI)	1.00 (ref)	1.81 (1.41–2.33)	<0.0001	1.00 (ref)	1.85 (1.38–2.49)	<0.0001	
Multivariate-adjusted HR[b] (95% CI)	1.00 (ref)	1.79 (1.39–2.30)	<0.0001	1.00 (ref)	1.73 (1.29–2.33)	0.0003	0.54
Laparoscopically confirmed endometriosis	**Age <35 years**			**Age 35–39 years**			***P*-het**
	No	**Yes**	***P*-value**	**No**	**Yes**	***P*-value**	
Cases, *n*	2896	114		1533	69		
Person-years	156 338	3038		197 151	5691		
Age-adjusted HR (95% CI)	1.00 (ref)	2.12 (1.76–2.56)	<0.0001	1.00 (ref)	1.56 (1.23–1.99)	0.0003	
Multivariate-adjusted HR[a] (95% CI)	1.00 (ref)	2.11 (1.74–2.54)	<0.0001	1.00 (ref)	1.57 (1.23–2.00)	0.0003	
Multivariate-adjusted HR[b] (95% CI)	1.00 (ref)	1.77 (1.46–2.14)	<0.0001	1.00 (ref)	1.20 (0.94–1.53)	0.14	0.008
Laparoscopically confirmed endometriosis	**BMI <25 kg/m^2**			**BMI 25+ kg/m^2**			***P*-het**
	No	**Yes**	***P*-value**	**No**	**Yes**	***P*-value**	
Cases, *n*	3092	145		1326	37		
Person-years	239 447	5626		113 012	3068		
Age-adjusted HR (95% CI)	1.00 (ref)	2.44 (1.98–3.00)	<0.0001	1.00 (ref)	1.33 (0.85–2.09)	0.21	
Multivariate-adjusted HR[a] (95% CI)	1.00 (ref)	2.46 (1.99–3.03)	<0.0001	1.00 (ref)	1.28 (0.82–2.00)	0.28	
Multivariate-adjusted HR[b] (95% CI)	1.00 (ref)	1.96 (1.59–2.42)	<0.0001	1.00 (ref)	1.30 (0.83–2.05)	0.25	0.02

[a]Adjusted for age (months), age interaction term (except for age stratified analysis), questionnaire cycle, age at menarche, menstrual cycle length between ages 18 and 22 years, current BMI, total physical activity, smoking status, cumulatively updated average AHEI diet score.
[b]Additionally adjusted the multivariate model for parity (number of children).

primary compared with secondary infertility (HR of 3.68 versus 2.57 in multivariate models, respectively; *P*-interaction = 0.09). Similarly, when we stratified our sensitivity analysis by age or BMI, we observed stronger risks when compared with the main analysis, and heterogeneity between strata remained statistically significant (Table IV). If we expanded our exposure period even further, assuming endometriosis had been present at least 4 years prior to the endometriosis report, results were similar and perhaps slightly stronger (data not shown). When infertile women with a concurrent endometriosis diagnosis were censored instead, endometriosis still conferred a 2-fold risk of infertility in the fully adjusted model (data not shown).

Discussion

In the current study, we investigated the prospective relationship between a history of laparoscopically confirmed endometriosis and infertility risk, in a large, well-characterized cohort of women. Women with endometriosis had ~2-fold greater risk of subsequent infertility after accounting for all established infertility risk factors. Further, this relationship was apparent only among women <35 years of age and those of normal weight (BMI < 25 kg/m^2). When we classified women reporting endometriosis on the same questionnaire as their infertility, with the possibility that both diagnoses were the result of the same infertility investigation, from unexposed to exposed, we observed a substantial increase in the effect estimate.

For almost a century, endometriosis has been cited as the most commonly observed disease among infertile women undergoing laparoscopic examination, leading to the perception of causality (Wheeler, 1989). Consistent with ranges reported by prior studies (Wheeler, 1989; Tanahatoe *et al.*, 2003; Giudice and Kao, 2004; Missmer *et al.*, 2004; Giudice *et al.*, 2010), we observed a 6% prevalence of laparoscopically confirmed endometriosis in our population of premenopausal married women <40 years of age, and 16% among the women subsequently diagnosed with incident infertility. Endometriosis as the underlying cause of infertility is less debatable in advanced endometriosis cases where pelvic anatomy is distorted, blocking proper release and transport of oocytes. However, the evidence to support a causal relationship of mild stage disease with infertility is weak (Witz and Burns, 2002). Furthermore, the current staging system does not distinguish between potentially pertinent subtypes of superficial versus infiltrating disease (Hackethal *et al.*, 2010). Nevertheless, most research has focused on potential biologic mechanisms (Wheeler, 1989), including poor oocyte quality, endometrial dysfunction and a chronic inflammatory embryo toxic environment, none of which are currently well established (Witz and Burns, 2002).

Of the few studies evaluating the endometriosis–infertility relationship, all of which were cross-sectional with respect to endometriosis and infertility diagnosis, estimates have ranged from no association to a 20-fold risk (Strathy *et al.*, 1982; Balasch *et al.*, 1996; Herbert *et al.*, 2009; Paris and Aris, 2010; Collazo *et al.*, 2012). Our observed estimate

Table IV History of laparoscopically confirmed endometriosis and risk of incident infertility among married women in Nurses' Health Study II from 1989 to 2005, includes women concurrently diagnosed with endometriosis and infertility.

Laparoscopically confirmed endometriosis	Total population			Primary infertility			Secondary infertility			*P*-het
	No	Yes	*P*-value	No	Yes	*P*-value	No	Yes	*P*-value	
Cases, *n*	4206	406		1968	265		2238	141		
Person-years	351 318	10 901		42 135	1880		309 183	9021		
Age-adjusted HR (95% CI)	1.00 (ref)	4.02 (3.55–4.55)	<0.0001	1.00 (ref)	3.70 (3.17–4.33)	<0.0001	1.00 (ref)	2.65 (2.12–3.32)	<0.0001	
Multivariate-adjusted HR[a] (95% CI)	1.00 (ref)	3.23 (2.85–3.67)	<0.0001	1.00 (ref)	3.68 (3.15–4.31)	<0.0001	1.00 (ref)	2.57 (2.06–3.22)	<0.0001	0.09
Laparoscopically confirmed endometriosis				**Age <35 years**			**Age 35–39 years**			***P*-het**
				No	**Yes**	***P*-value**	**No**	**Yes**	***P*-value**	
Cases, *n*				2731	279		1475	127		
Person-years				155 399	3978		195 917	6923		
Age-adjusted HR (95% CI)				1.00 (ref)	4.02 (3.55–4.55)	<0.0001	1.00 (ref)	2.44 (2.03–2.92)	<0.0001	
Multivariate-adjusted HR[a] (95% CI)				1.00 (ref)	3.21 (2.83–3.64)	<0.0001	1.00 (ref)	1.83 (1.52–2.20)	<0.0001	<0.001
Laparoscopically confirmed endometriosis				**BMI <25 kg/m^2**			**BMI 25+ kg/m^2**			***P*-het**
				No	**Yes**	***P*-value**	**No**	**Yes**	***P*-value**	
Cases, *n*				2922	315		1273	90		
Person-years				237 944	7127		112 358	3722		
Age-adjusted HR (95% CI)				1.00 (ref)	4.46 (3.87–5.14)	<0.0001	1.00 (ref)	2.95 (2.25–3.87)	<0.0001	
Multivariate-adjusted HR[a] (95% CI)				1.00 (ref)	3.42 (2.96–3.95)	<0.0001	1.00 (ref)	2.72 (2.06–3.59)	<0.0001	0.05

[a]Adjusted for age (months), age interaction term (except for age stratified analysis), questionnaire cycle, age at menarche, menstrual cycle length between ages 18 and 22 years, current BMI, total physical activity, smoking status, cumulatively updated average AHEI diet score, parity (number of children).

is most consistent with a retrospective cross-sectional Canadian registry-based study, which observed ~2-fold increased infertility risk associated with clinical or laparoscopically confirmed endometriosis (Paris and Aris, 2010).

Cross-sectional analyses cannot account for temporality, and thus are susceptible to the bias that may be introduced when endometriosis is diagnosed as a result of an infertility investigation. We were able to simulate the potential impact on the HR from this bias by counting women as 'exposed' when reporting endometriosis and infertility on the same questionnaire. Doing so gave a much higher HR, similar to what was observed in an Australian longitudinal study in which information on endometriosis was retrospectively collected (Herbert *et al.*, 2009).

Our stratified multivariate analyses indicated that the greater risk of infertility after laparoscopically confirmed endometriosis may only be significantly elevated among lean women and those <35 years of age. Excess adiposity alters sex steroid hormone bioavailability and contributes to metabolic abnormalities, which may disrupt folliculogenesis and endometrial receptivity (Talmor and Dunphy, 2015). After 35 years of age, ovarian reserve of quality oocytes is rapidly depleted and risk of other disorders increase, becoming more prominent causes of infertility (American College of Obstetricians and Gynecologists Committee on Gynecologic Practice and Practice Committee, 2014). For women over 35 years, clinicians may prioritize assisted reproductive treatments over laparoscopy, leaving endometriosis undiagnosed. However, this bias would be limited to our sensitivity analysis (Table IV), would drive risk estimates among women aged 35–39 years toward the null, and may explain some of the greater heterogeneity in infertility risk by age when infertile women with 'concurrent' endometriosis were considered as exposed cases.

Greater awareness of endometriosis among young adults and clinicians, leading to increased screening practices, could be contributing to the growing prevalence of diagnosis of the disease among young women and represent an alternate explanation as to why risk appears stronger among women <35 years of age (Paris and Aris, 2010). Likewise, the perception that endometriosis is more strongly associated with primary versus secondary infertility (Strathy *et al.*, 1982), may have contributed toward greater bias in the risk estimate among nulliparous women when we included infertile women with concurrently diagnosed endometriosis as exposed cases.

This study has many important strengths including the longitudinal study design of a well-characterized cohort in which risk factors and medical conditions were regularly updated over follow-up and determined to be highly valid. Additionally, our wealth of data provided the means to assess a number of potential confounders and effect modifiers and mediation by parity. The prospectively collected information eliminates recall bias. The unique features of our cohort allowed us to assess whether laparoscopically confirmed endometriosis preceded an infertility diagnosis, a temporal relationship necessary for inferring causality. We were also able to demonstrate the extent to which diagnostic bias may influence the association between endometriosis and infertility.

We recognize that our study has several limitations that must be addressed. Owing to the frequency of our questionnaires, it is possible that some proportion of women who received an endometriosis

diagnosis prior to incident infertility but within the same questionnaire cycle were incorrectly classified as 'unexposed' incident infertility cases in our main analysis. This systematic misclassification would bias our main analysis results by reducing risk estimates toward the null, with the magnitude of bias dependent on the proportion of women misclassified. However, given that the vast majority of women eligible for this study with both endometriosis and incident infertility reported an endometriosis diagnosis either on the same questionnaire or on questionnaires subsequent to the infertility report, we suspect that the proportion of misclassified women is likely to be small and, therefore, the potential underestimation caused by the bias is negligible.

Without information on participants' intention to conceive, our cohort includes women not at risk of infertility. In the absence of this information, the composition of our cohort—married, non-Hispanic white and highly educated—reflects some of the strongest predictors of planning a pregnancy (Chandra *et al.*, 2014), and therefore unsuccessful attempts are more likely to be recognized. We additionally restricted our main analysis to premenopausal married women <40 years of age to reduce the overall proportion of women who are not at risk for infertility. Even so, our estimates remained the same in sensitivity analyses that included unmarried premenopausal women <40 years of age. Women in our cohort with undiagnosed asymptomatic endometriosis will be misclassified as unexposed. Given the low prevalence of undiagnosed endometriosis in the general population (<2% reported by Zondervan *et al.* (2002)) and the prospective nature of this study, we expect this to be a very small percentage and will be diluted among the >50 000 women accurately characterized as endometriosis-free. Further, any misclassification should be non-differential with respect to the infertility outcome and the large number of truly unexposed women in the cohort minimizes bias toward the null.

In the absence of an infertility investigation, the women diagnosed with laparoscopically confirmed endometriosis are symptomatic. Thus, most women in our main analysis with laparoscopically confirmed endometriosis were diagnosed because of pain. If etiology differs between pain symptomatic versus asymptomatic disease, then our infertility risk estimates may be generalizable only to women with symptomatic endometriosis. While we do not have information on the severity or location of their endometriotic lesions, prior studies suggest the staging system does not correlate with pain or infertility (Barbieri and Missmer, 2002). We also lack information on the treatment or recurrence of endometriosis, which may mediate infertility risk. However, current evidence suggests hormonal medical treatment for endometriosis has no effect on infertility while surgery provides only ~8% improvement in cumulative pregnancy rate among Stage I–II endometriosis patients. High-quality data regarding reproductive benefit of surgery for more advanced endometriosis is lacking (Vercellini *et al.*, 2014).

Finally, the participants in our cohort are female registered nurses, potentially limiting the generalizability of our results. Given their general interest in health, greater health evaluation seeking behaviors, enhanced reporting accuracy of exposures and health outcomes, and high follow-up rate, nurses were specifically chosen to maximize the cohort's internal validity. If the underlying biology of endometriosis and infertility is similar across ethnic groups, region and social class, then our observations will apply more broadly.

In conclusion, we observed a 2-fold increased risk of subsequent infertility among women with a prior history of laparoscopically confirmed endometriosis that was limited to women age <35 years and/or with BMI <25 kg/m^2. We demonstrated the potential impact of diagnostic bias on the magnitude of risk when we included infertile women with concurrently diagnosed endometriosis as exposed cases, as has been done in all prior studies. Additional prospective studies are warranted to confirm our findings and research is needed to determine whether etiology of symptomatic versus asymptomatic endometriosis differs and how this may affect infertility risk.

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Authors' roles

S.A.M. led the conceptualization and supervision of the analysis. J.P. performed the analyses and drafted the manuscript. All authors critically revised and approved the final manuscript.

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Conflict of interest

None declared.

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